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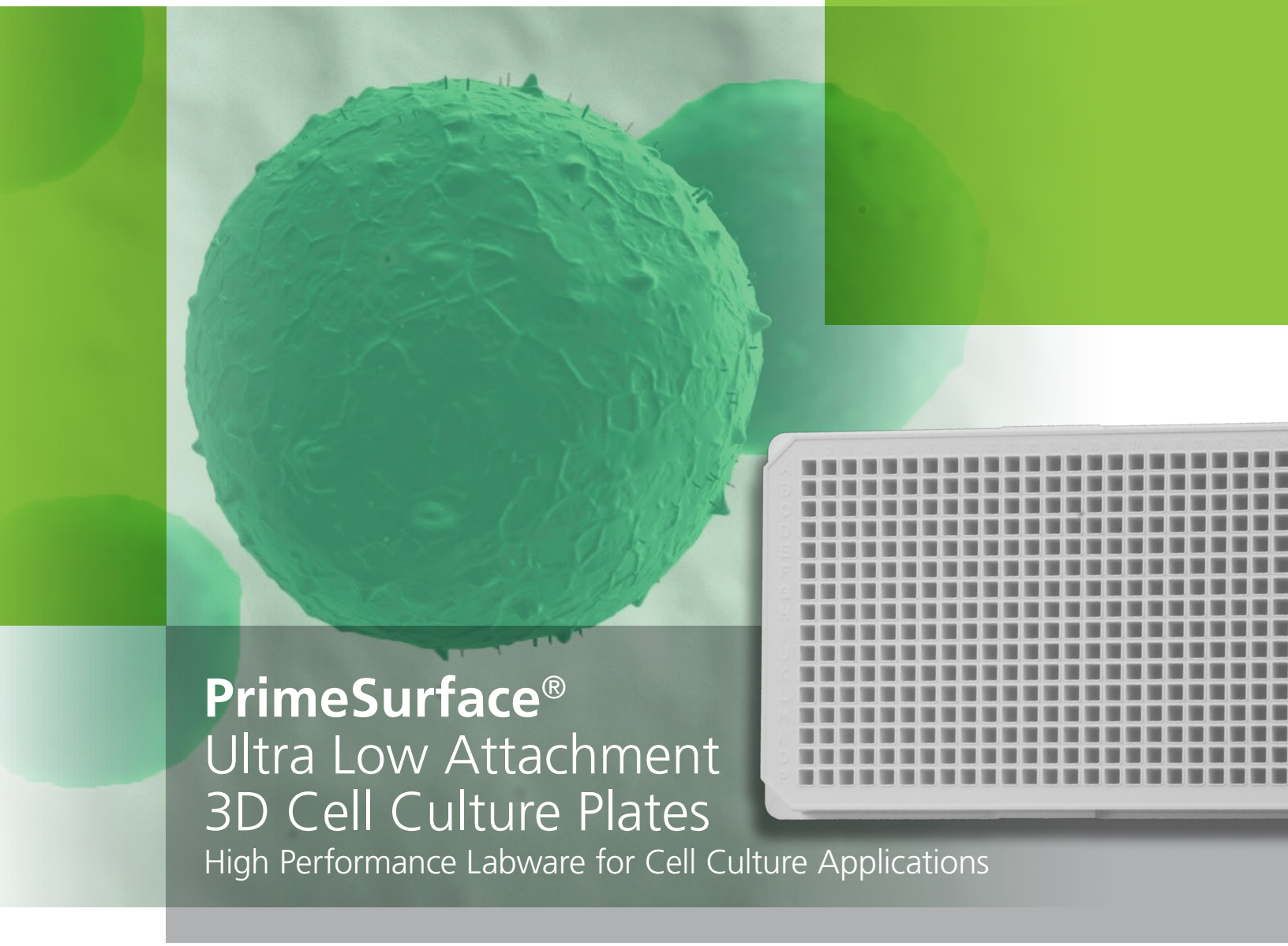

PrimeSurface®
Ultra Low Attachment 3D Cell Culture Plates

High Performance Labware for Cell Culture Applications

Streamlined High Throughput Drug Screening with PrimeSurface White 384 Well 3D plates

Stem Cell Research | Drug Discovery and Development | Tissue Engineering | Regenerative Medicine

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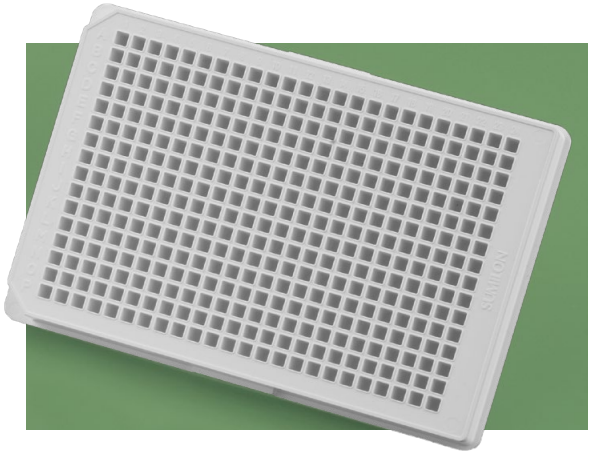
PrimeSurface

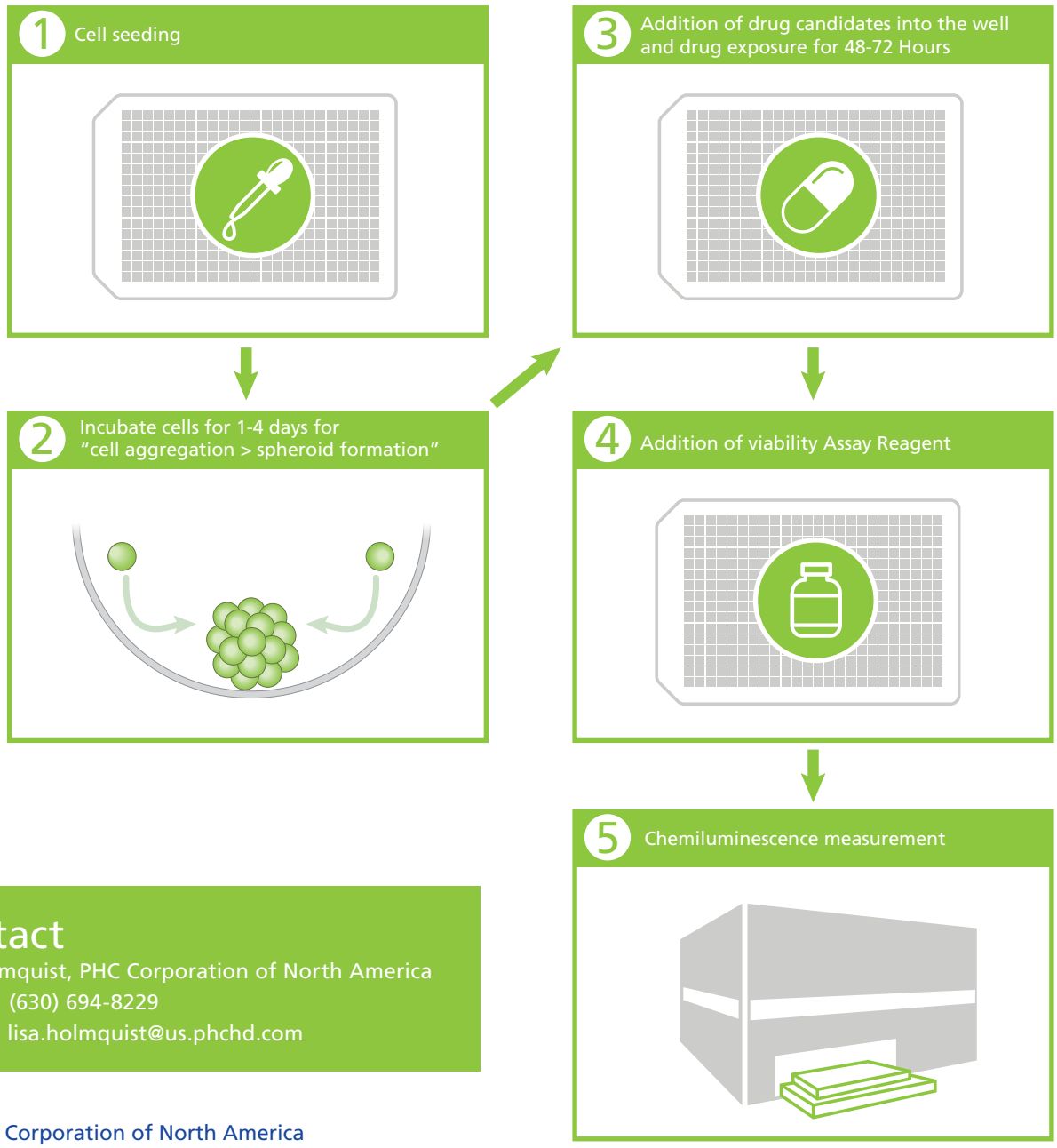


Using PrimeSurface white plates, cancer spheroid cells can be formed and tested for drug screening using luminescence viability assays in the same well without the need for sample transfer.

This streamlined process shortens the experimental steps and time required in drug testing, and reduces the risk of damaging valuable samples during handling and transfer.

In this technical note, we demonstrate cell viability of 4 different tumor cells and the streamlined method of intracellular ATP measurement using CellTiter Glo® (Promega Co., Ltd).

Experimental Workflow



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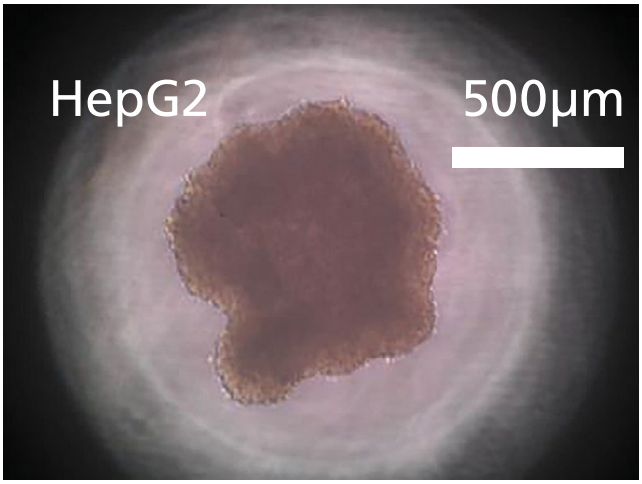
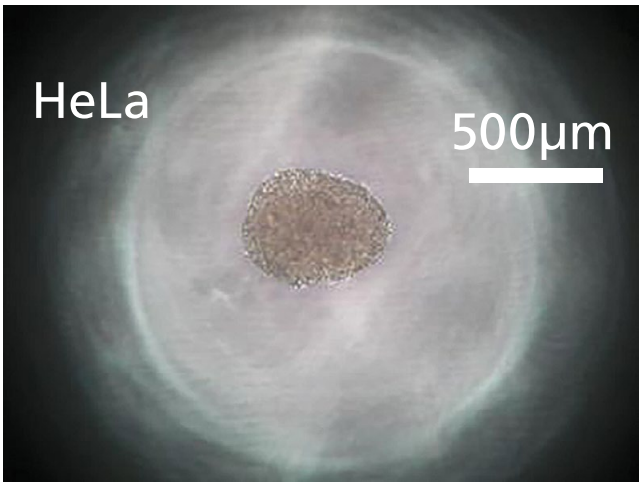
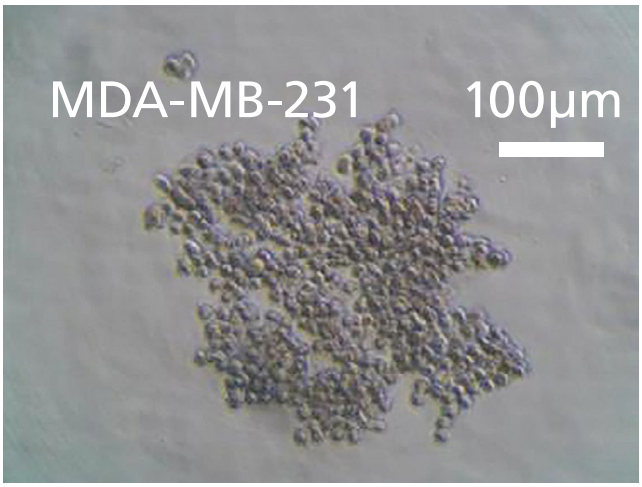
Experimental Examples

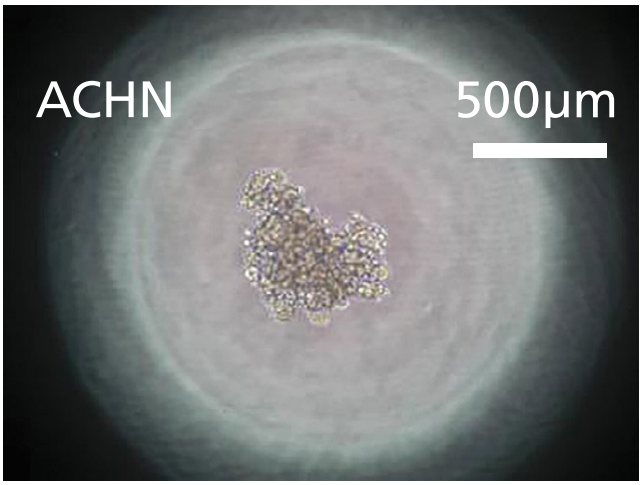
Different types of Cancer cell lines can form tight, compact or loose spheroids. Four types of cells, shown below serve as representative cases for spheroid formation. Using CellTiter Glo, a homogeneous “add-mix-measure” reagent results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present which is directly proportional to the number of cells present in culture.

Toughness	Cells	Origin
Tight	HepG2	Human Hepatic Cancer Cell Line
Compact	HeLa	Human Cervical Cancer Cell Line
Loose	MDA-MB-231	Human Breast Cancer Cell Line
Loose	ACHN	Human Renal Adenocarcinoma Cell Line
MS-9096VZ	PrimeSurface 96V	96
MS-9384UZ	PrimeSurface 384U	384
MS-9384WZ	PrimeSurface 384W	384









Materials

Culture Medium: RPMI1640 (+10%FBS +1%Penicillin-Streptomycin Mixed Solution)
CellTiter Glo Luminescent Cell Viability Assay Cat. No. G7572 (Promega Co., Ltd.)
PrimeSurface 384 well clear plate (MS-9384UZ, Sumitomo Bakelite Co., Ltd.)
PrimeSurface 384 well white plate (MS-9384WZ, Sumitomo Bakelite Co., Ltd.)

Equipment

Plate Reader: Fusion α-FP (Perkin Elmer Co., Ltd)

[Methods]

Cells were seeded in PrimeSurface 384 well white plate (Cat. No. MS-9384WZ) with a density of 250, 500 and 1000 cells/well in 25µL of culture medium*[1]. Cells were incubated at 37°C / 5°C CO_2. Luminescent intensities were measured every two days after addition of 25µL volume of CellTiter Glo reagent and ten minutes standing at R.T.*[2]

*1: Based on the type of cells, number of cells, and culture medium, the amount of culture medium may need to be adjusted.
*2: If cell solubility is poor, dissolve the cells using a shaker.

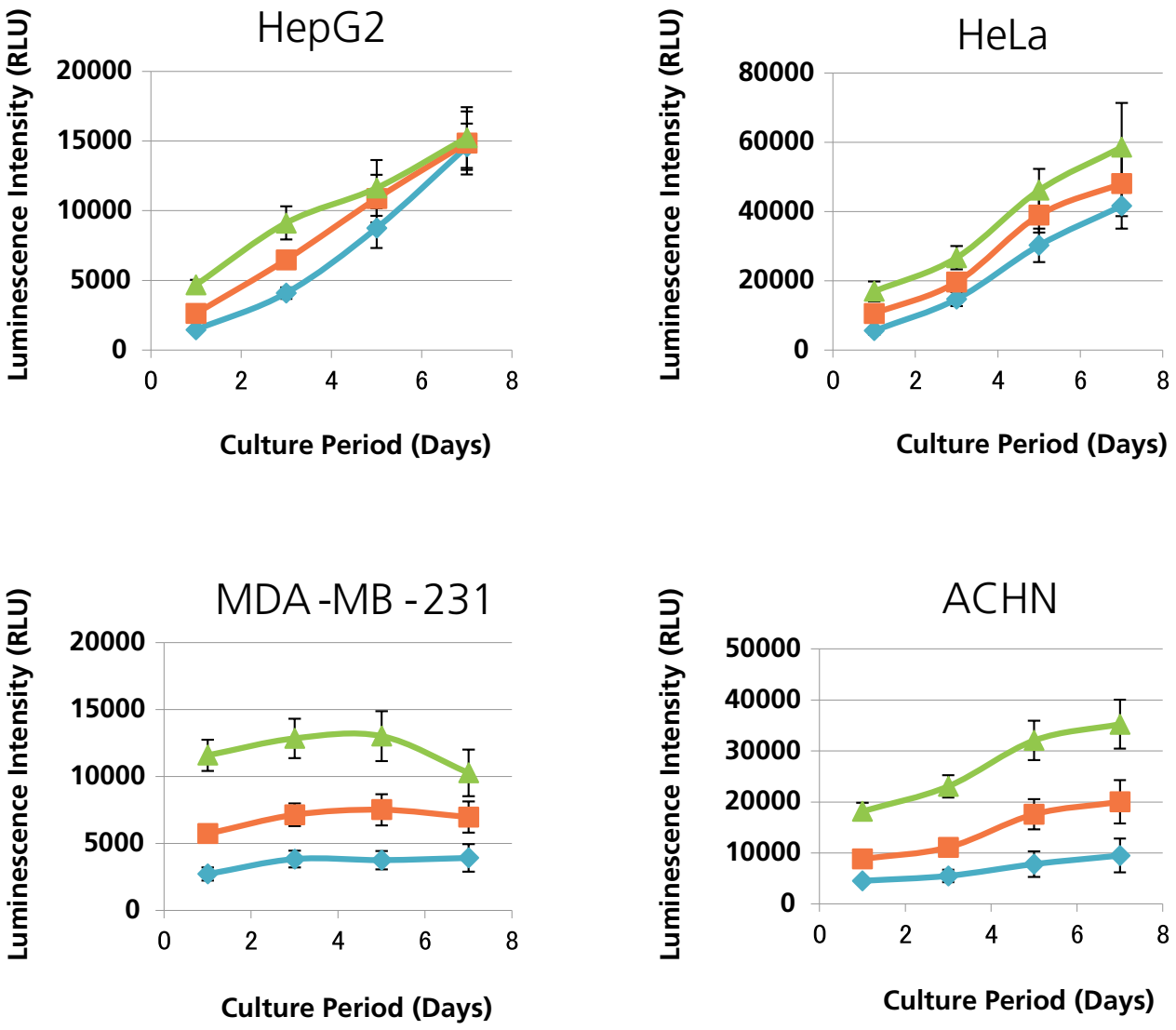


Fig. 1 Cell proliferation curve of HepG2, HeLa, MDA-MB-231 and ACHN cells

(◆250cells/well, ■500cells/well, ▲1000cells/well)

The amount of ATP increased in the case of HepG2, HeLa, and ACHN cells with all cell seeding densities over the 7 days culture period. On the other hand, MDA-MB-231 did not show much increase in the ATP amount.

Data above represent typical values.

[Methods]

1) Cells were seeded with 25μL/well media in PrimeSurface 384 well white plate (MS-9384WZ) as stated in the "Experimental Examples" method. First column and last column served as the control wells with no cells and culture medium only.

2) The intracellular ATP amount was measured at Day 5 and 25 μL of CellTiter Glo reagent was added to each well.

3) Z'-factor values were calculated as 100% and 0% and respectively, in wells with and without cells.

		HepG2		HeLa		MDA-MB-231		ACHN	
	Cells/Well	All Well	Inner Well	All Well	Inner Well	All Well	Inner Well	All Well	All Well
PrimeSurface 384 well white plate	250	0.58	0.67	0.58	0.56	0.41	0.44	0.41	0.40
	500	0.51	0.54	0.59	0.59	0.55	0.60	0.46	0.45
	1000	0.50	0.49	0.52	0.51	0.62	0.70	0.67	0.67

$$\text{Z-facor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}.$$

Inner well : includes all inner wells and excludes outermost peripheral wells in the plate

Data above represent typical values

HepG2 and HeLa cells, having a tight spheroid formation ability, showed Z'-factors values higher than 0.5. MDA-MB-231 and ACHN cells, having a loose spheroid formation ability also showed Z'-factors values higher than 0.4

σ_p: SD of positive samples, σ_n: SD of positive samples

μ_p: mean values of positive samples, μn: mean values of positive samples

Z-Factor values:

1 > Z > 0.5: Excellent assay, 0.5 > Z > 0: Allowable assays, Z < 0: Unallowable assays

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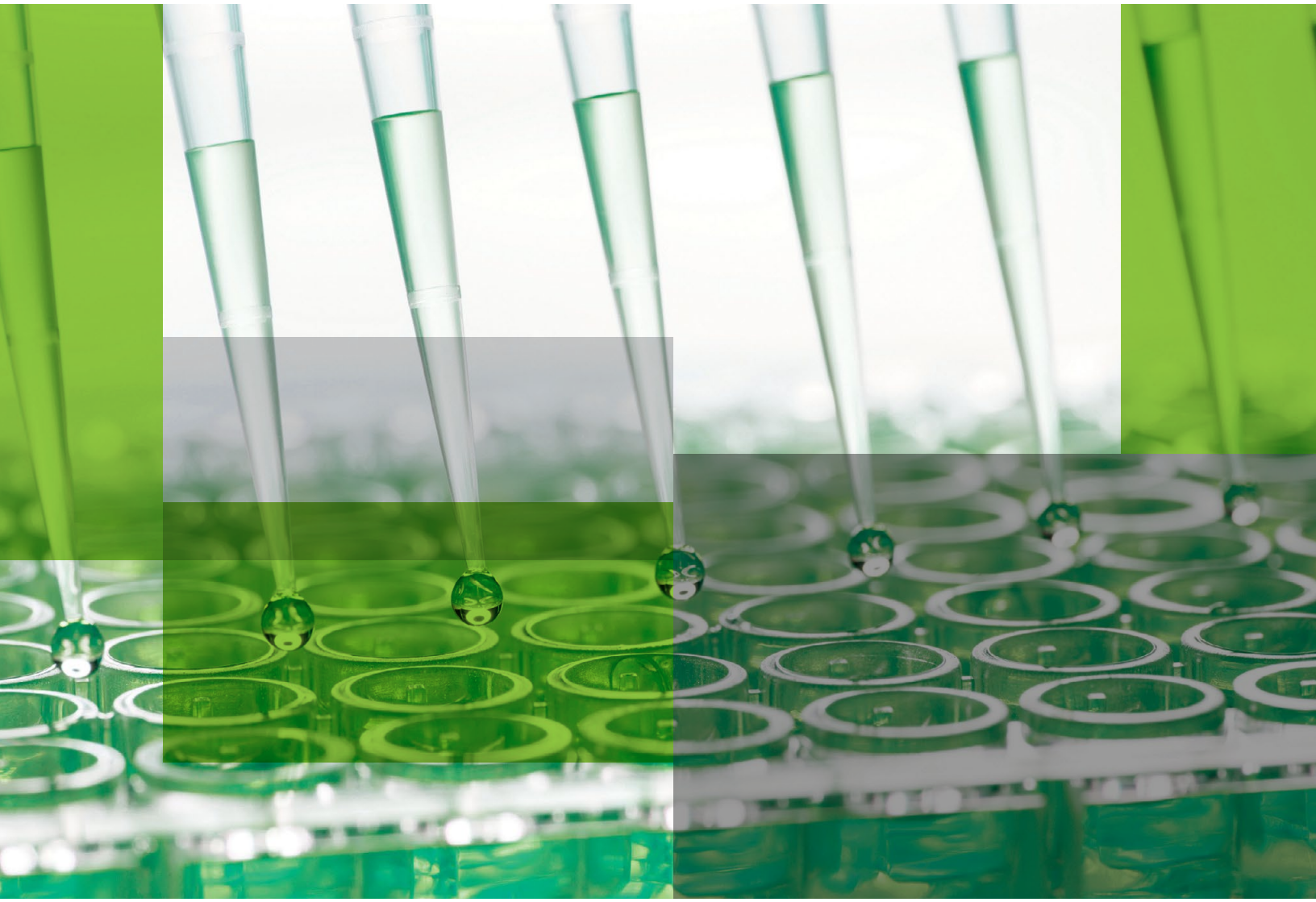
Additional Products

Complementary product lines under the PHCbi brand include the space saving and energy efficient VIP® ECO, VIP Series and TwinGuard® ultra-low temperature freezers, cryogenic and biomedical freezers, pharmacy and high performance refrigerators, cell culture CO_2 and multigas incubators, programmable heated and refrigerated microbiological incubators, Class II, Type A2 biological safety cabinets, portable autoclaves, cell processing work stations and Drosophila/Plant Growth Chambers. For more information, please call PHC Corporation of North America at 800-858-8442, email info@us.phchd.com or visit http://www.phchd.com/us/biomedical.



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